

## Effects of various continuous cropping times on soil nematode structure in cotton fields of Xinjiang, China

Efectos de varios tiempos de cosecha continua en la estructura de los nematodos del suelo en los campos de algodón en Xinjiang, China

Li XL<sup>1,2</sup>, YC Wang<sup>3</sup>, CA Busso<sup>4</sup>, JS Xiang<sup>1</sup>, AM Zhang<sup>1</sup>, YW Qu<sup>1</sup>, Y Liu<sup>2</sup>

**Abstract.** Long-term continuous cropping of cotton had led to substantial agricultural losses. However, continuous cotton cropping could maintain high crop yields for many years in some areas. The composition and structure of soil nematode communities were investigated to explore the effect of continuous cropping and soil depths on these communities. Soil samples were collected at two soil depths (0-20 cm and 20-40 cm) from cotton fields with a history of 5, 10, 15 or 20 years of continuous cotton cropping in the Karamay region. The results showed that 36 genera were found. Significant differences in the numbers of total nematodes and trophic groups were observed among continuous cropping times. The highest number of total nematodes was observed at 20-40 cm depth under continuous 10-cropping years, and the lowest number at 20-40 cm depth under continuous 5-cropping years. There were significant soil depth effects on the density of fungivores and plant parasites. Continuous cropping effects on soil nematode communities could be reflected by values of ecological indexes. Continuous cropping times, soil depths and their interaction significantly influenced  $H'$ ,  $\lambda$ , WI, PPI (index of plant parasites), MI (maturation index excluding plant parasites), PPI/MI, F/B and NCR. The Shannon index ( $H'$ ) was higher in the 10-year cotton field than in the other-year fields. The Simpson index ( $\lambda$ ) was the lowest in the 10-year cotton field. The lowest value of WI (index of Wasilewska) appeared at the 20-year cotton field, which had the majority of plant parasites. Increases of cotton cropping times determined a decreased trend of NCR [ $=B/(B + F)$ ], and an increased trend of F/B [F and B represent the consumer abundance of fungi (F) and bacteria (B)]. The bacterial decomposition pathway was more important in the 5-year situations, and the fungal decomposition pathway was more important in the 20-year situations. Nematode analysis showed that changes

**Resumen.** Cosechas continuas a largo plazo de algodón han conducido a pérdidas agrícolas substanciales. Sin embargo, la cosecha continua de algodón podría mantener altos rendimientos del cultivo durante varios años en algunas áreas. La composición y estructura de las comunidades de nematodos del suelo se investigaron para explorar el efecto de cosechas continuas y profundidades del suelo en estas comunidades. Se recolectaron muestras de suelo a dos profundidades (0-20 y 20-40 cm desde la superficie del suelo) en campos de algodón con una historia de 5, 10, 15 ó 20 años de cosechas continuas en la región de Karamay. Se determinaron 36 géneros de nematodos. Hubo diferencias significativas en el número total de nematodos y grupos tróficos entre años de cosecha continua. El mayor número de todos los nematodos se observó a 20-40 cm de profundidad del suelo bajo cosechas continuas de algodón de 10 años, y el menor número a 20-40 cm de profundidad bajo cosechas continuas de 5 años de dicho cultivo. Hubo efectos significativos de las profundidades de suelo en la densidad de fungívoros y parásitos vegetales. Los efectos de cosechas continuas en las comunidades de nematodos del suelo se podrían observar por valores de índices ecológicos. Años de cosechas continuas, profundidades del suelo y su interacción influenciaron significativamente  $H'$ ,  $\lambda$ , WI, PPI (índice de parásitos vegetales), MI (índice de madurez, excluyendo los parásitos vegetales), PPI/MI, F/B [F y B representan la abundancia de consumidores de hongos (F) y bacterias (B)] y NCR ( $=B/(B + F)$ ). El índice de Shannon ( $H'$ ) fue mayor en el campo de algodón de 10 años de cosecha continua que en los otros campos estudiados. El menor índice de Simpson ( $\lambda$ ) se observó en el campo de algodón de 10 años. El valor más bajo del índice de Wasilewska (WI) pareció estar en el campo de algodón de 20 años, el que tuvo la mayoría de los parásitos vegetales. Incrementos en los años de cosecha de algodón determinaron una tendencia descendiente de NCR, y una tendencia ascendente de F/B. La senda de descomposición bacteriana fue más importante

<sup>1</sup>Chifeng University, Chifeng 024000, China.

<sup>2</sup>Xinjiang Academy of Agricultural and Reclamation Science, Shihezi 832000, China.

<sup>3</sup>Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China.

<sup>4</sup>Departamento de Agronomía-CERZOS (CONICET: Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina), Universidad Nacional del Sur (UNSur), San Andrés 800, 8000 Bahía Blanca, Argentina.

Address correspondence to: Yongcui Wang, e-mail: yongcuiwang@iac.ac.cn; Xiaolan Li, e-mail: lixiaolan97@163.com; Tel.: +86-24-83970442; fax: +86-24-83970339.

Received 11.IV.2016. Accepted 30.VII.2016.

of soil nematode communities and trophic groups could indicate changes in the soil environment and nematode community structure with changes of continuous cropping times.

**Keywords:** Nematode abundance; Continuous planting; Ecological indexes; Trophic groups; Soil depth.

en las situaciones de 5 años, y la senda de descomposición fúngica fue más importante en las situaciones de 20 años. El análisis de nematodos mostró que los cambios en las comunidades de nematodos del suelo y grupos tróficos podrían indicar cambios en el ambiente del suelo y la estructura de las comunidades de nematodos con cambios en el tiempo de cosechas continuas.

**Palabras clave:** Abundancia de Nematodos; Plantación Continua; Índices Ecológicos; Grupos tróficos; Profundidad del suelo.

## INTRODUCTION

Xinjiang is China's most important cotton planting area. It accounts for more than half of the total national cotton production (Jin, 2008). The planting area of cotton has reached more than one third of the total cultivated area in the Xinjiang region (Statistical Bureau of Xinjiang, 2010). Long-term, continuous production of cotton is very common. As a result, poor growth potential, serious diseases and pests, pesticide and fertilizer pollution, high costs and low economic efficiency are gradually increasing on producing this crop (Ni et al., 2002).

Nematodes are one of the most abundant groups of soil invertebrates (Fu et al., 2000). They occupy central positions in the soil food web, and correlate with soil ecological processes such as nitrogen cycling and plant growth (Neher, 2001). Soil nematode communities and their structural changes are one of the best biological tools for assessing soil processes and plant conditions in terrestrial ecosystems (Wang et al., 2009; Pen-Mouratov et al., 2010). Nematode communities are quite susceptible to disturbances across several types of land uses in recent studies (Wu et al., 2002; Ferris & Matute, 2003; Liang et al., 2005, 2007; Okada & Harada, 2007; Powell, 2007). Farmland ecosystems are important terrestrial ecosystems intensively affected by human activities. Agricultural practices such as soil cultivation, monocultures, and the application of chemicals including fertilizers, herbicides, insecticides, and nematicides can alter nematode populations (Wu & Shi, 2011). A variety of statistical techniques or indices have been developed to describe environmental disturbances using nematode species, trophic structures or life strategies (Yeates et al., 1997, 2003; Bongers & Ferris, 1999; Ruess et al., 1999; Ferris et al., 2001).

The purpose of this research was to determine the effect of continuous cropping on soil nematode communities in a northern Xinjiang farmland. The objectives of this study were: to (1) assess the distribution of nematode populations in planting areas exposed to continuous cropping with cotton for different time periods, (2) evaluate the nematode population distribution on different trophic groups in these soils, and (3) assess the effects of nematode ecological indices on different time periods of continuous cotton-cropping.

## MATERIALS AND METHODS

**Study sites.** This study was conducted at continuously cropped cotton fields in the Karamay region in Xinjiang (44°39'-46°8'N, 84°44'-86°1'E). This area has a temperate continental climate. Altitudinal gradient was from 270 to 500 m.a.s.l. Mean annual temperature was about 8.6 °C, and mean annual precipitation was about 108.9 mm. Evaporation was high (2692 mm), mean relative humidity was 60%, and there was plentiful luminous energy.

**Soil sampling.** Continuously cropped cotton fields with the same management were selected. Continuous cropping times included 5, 10, 15 or 20 years. Each cotton field was larger than 5 ha. For each field, five replicate plots (2m×2m for each) were selected following an S-shaped pattern. The minimal distance between any two plots was about 50 m. In each plot, five soil cores using an X-shaped pattern were taken to make a composite soil sample. Soil samples were obtained using a soil corer (5 cm in diameter) at 0-20 cm and 20-40 cm depths in September 2013. A total of 40 soil samples were then obtained (4 continuous cropping times × 5 replicate plots/cropping time × 2 soil depths/cropping time/soil core). Visible roots and organic residues were removed from the samples. Thereafter, all samples were stored individually in plastic bags at a constant temperature of 4 °C, and processed within one week.

**Extraction and identification of soil nematodes.** Nematodes were extracted from 100 g fresh soil by a modified cotton-wool filter method (Liang et al., 2009). Density of nematodes was expressed as individuals per 100 g dry soil. At least 100 nematodes from each sample were identified to a genus level using an inverted compound microscope (Jairajpuri & Ahmad, 1992; Ahmad & Jairajpuri, 2010). Nematodes were divided into 4 trophic groups according to their feeding habit (Yeates et al., 1993): bacterivores (BF), fungivores (FF), plant parasites (PP) and omnivores-predators (OP). Nematodes were also allocated to colonization-persistence (c-p) classes following Bongers (1990): the colonization-persistence scale ranged from 1 (colonizers) to 5 (persisters). The c-p values of 1-5 reflect the perceived gradi-

ent among nematodes from colonizing r-strategists with unstable populations to persisting k-strategists with relatively stable populations.

**Nematode community analyses.** To characterize the soil nematode community, the following indexes were calculated: (1) Relative density: it is the percent composition of an organism of a particular kind relative to the total number of organisms in the area (Walag & Canencia, 2016). Soil nematode genera represent organisms in ecological studies of nematodes. The relative density of any genus was calculated as the proportion from the 100 nematodes identified per sample; more than 5% values were dominant genera; (2) Species richness ( $S$ ): the number of soil nematode genera; (3) Shannon-Weaver diversity index ( $H'$ ):  $H' = -\sum P_i \times \ln P_i$ , where  $P_i$  is the proportion of individuals in the  $i^{\text{th}}$  genera (Shannon, 1948); (4) Simpson's index,  $\lambda = \sum (P_i)^2$ , where  $P_i$  is the proportion of individuals in the  $i^{\text{th}}$  genera (Simpson, 1949); (5) Wasilewska index (WI),  $WI = (F + B)/P$ , where F, B and P are the abundances of bacterivores, fungivores and plant parasites, respectively (Wasilewska, 1997); (6) Maturity index (MI) (excluding plant parasites),  $MI = \sum [v(i) \times f(i)]$ , where  $v(i)$  is the colonizer-persister (c-p) value of genera  $i$ , and  $f(i)$  is the frequency of genera  $i$  in a sample (Bongers, 1990; Bongers & Bongers, 1998); (7) Plant-parasites index (PPI),  $PPI = \sum [v(i) \times f(i)]$ , where  $v(i)$  is the colonizer-persister (c-p) value of genera  $i$ , and  $f(i)$  is the proportion of genera  $i$  in a sample (Yeates et al., 1993); (8) The ratios of PPI to MI (PPI/MI), the MI and PPI are calculated using a c-p value that ranges from colonizer (c-p = 1 or 2) to persister (c-p = 5), with the index values representing life-history characteristics associated with r- and K-selection, respectively (Bongers et al., 1995); (9) The ratios of FF to BF (F/B), where F and B are abundances of bacterial- and fungal-feeders; the ratio value represents the decomposition pathway of the bacterial or fungal channel in detrital food webs (Twinn, 1974; Freckman & Ettema, 1993); (10) Nematode Channel Ratio [NCR =  $B/(B + F)$ ], where B and F are abundances of bacterial- and fungal-feeders (Yeates, 2003); NCR indicates the relative importance of the bacterial- and fungal-feeders in the decomposition channels.

**Statistical analyses.** All analyses were based on abundance and relative abundance of nematodes. When applying analysis of variance (ANOVA) with two factors (continuous cropping times and soil depths), the duration of continuous cropping effects was the main factor, and the interaction between factors could be evaluated. Differences at the  $P < 0.05$  or  $P < 0.01$  level were considered as statistically significant. Duncan's multiple comparison tests were carried out on means significantly different by ANOVA. All statistical analyses were performed using the statistical package SPSS version 16.0 (SPSS Inc., Chicago, IL).

## RESULTS

**Density and diversity of nematodes.** Thirty-six genera were observed in all nematode samples. The number of taxa ranged from 27 to 29 in the 5-year to 20-year cropped soils, respectively (Table 1); 15 genera were common in all situations. Some genera were only found in some continuous cropping times. The dominant genera were not perfectly consistent in different situations (Table 1). A relative density of more than 5% was considered as a dominant genus. Among the identified 36 nematode taxa in the soil at 0–40 cm depth, different genera were dominant at different planting years. These were the cases for *Rhabditidae* and *Rotylenchus* in the 5-year, *Tylenchus* in the 10-year, *Chilopacus*, *Aphelenchoides* and *Cervidellus* in the 15-year, *Pratylenchus* and *Psilenchus* in the 20-year, *Basiria* in the 10-, 15- and 20-years, and *Filenchus*, *Aphelenchus* and *Acrobeloides* in all continuous planting times (Table 1). The relative density of genera did exceed 5% in some but not all treatment combinations.

The numbers of genera were significantly higher at 0–20 cm than at 20–40 cm soil depth ( $P < 0.05$ ), but there were no significant differences among the various continuous cropping times, and the interaction continuous cropping time  $\times$  soil depth ( $P > 0.05$ ). The number of genera was 16–19 in the 0–20 cm soil depth, while it was 14–16 in the 20–40 cm soil depth (Table 1). The highest value was shown in the 10-year continuous planting (19 nematode genera) at 0–20 cm soil depth, and the lowest one in the 5-year continuous planting (14 nematode genera) at 20–40 cm soil depth (Fig. 1a).

The total density of soil nematodes varied among different continuous cropping times ( $P < 0.01$ ). The interactive effects of years and soil depths were significant to nematode density ( $P < 0.01$ ). The highest value was for the 10-year continuous planting (405 individuals/100 g dry soil) at 20–40 cm soil depth, and the lowest one for the 5-year continuous planting (223 individuals/100 g dry soil) at 20–40 cm soil depth (Fig. 1b). Soil depth had no effect on nematode density ( $P > 0.05$ ). Density of nematodes was significantly higher at 0–20 cm than 20–40 cm soil depth at the 5-year and 20-year continuous cropping. Nematode density was significantly lower at 0–20 cm than 20–40 cm soil depth in the 10-year and 15-year continuous cropping (Fig. 1b).

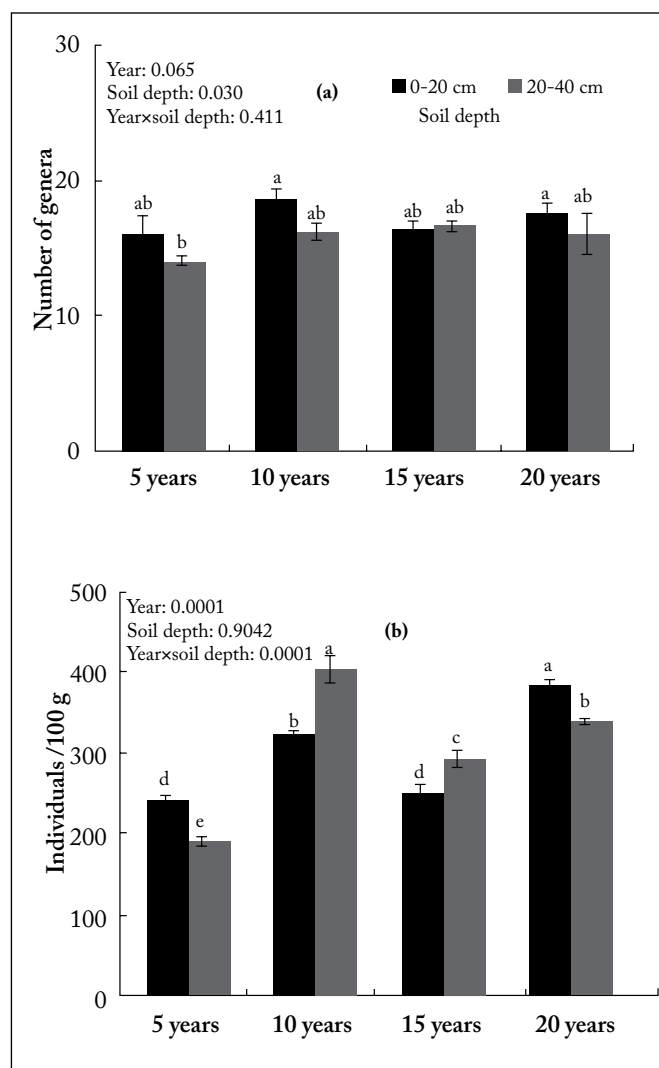
**Nematode trophic structure.** Density of bacterivores (BF), fungivores (FF) and plant parasites (PP) significantly varied among different continuous cropping times ( $P < 0.01$ ). The number of individuals in FF and PP were significantly affected by soil depths ( $P < 0.01$ ) (Table 2). The interaction effects between years and soil depths on all trophic groups were significant ( $P < 0.05$ ), except for density of PP ( $P > 0.05$ ) (Table 2). The highest density of BF and FF was observed in the 10-year continuous planting (150 and 109 individuals/100 g dry soil) at 20–40 cm soil depth (Table 2). The lowest den-

**Table 1.** Mean relative density (%) of nematode genera at different cotton continuous cropping times and soil depths.**Tabla 1.** Densidad relativa promedio (%) de géneros de nematodos en diferentes tiempos de cosechas continuas de algodón y profundidades del suelo.

Genus	5-year			10-year		15-year		20-year	
	c-p	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm	0-20 cm	20-40 cm
Bacterivores									
<i>Rhabditidae</i>	1	26.4	27.6	5.2	2.0	3.6	1.6	3.6	1.2
<i>Diplenteron</i>	1	0.0	0.0	0.0	0.6	0.0	0.2	0.2	0.0
<i>Cephalobus</i>	2	1.0	0.2	3.0	3.4	1.6	3.8	4.2	2.6
<i>Eucephalobus</i>	2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.2
<i>Acrobeles</i>	2	0.2	0.0	0.0	0.0	0.2	0.0	0.0	0.2
<i>Acrobeloides</i>	2	9.4	12.1	16.1	25.8	11.1	9.6	5.0	6.6
<i>Cervidellus</i>	2	1.8	0.8	2.8	1.4	5.2	5.2	0.2	0.0
<i>Chiloplacus</i>	2	1.6	5.0	2.6	4.0	9.8	6.6	2.2	1.6
<i>Achromadora</i>	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
<i>Prismatolaimus</i>	3	0.0	0.0	0.4	0.0	1.0	0.2	4.5	1.8
Fungivores									
<i>Filenchus</i>	2	7.4	7.3	14.9	13.0	20.5	16.3	4.8	9.2
<i>Aphelenchus</i>	2	16.8	4.0	9.2	5.4	8.8	8.8	17.3	6.2
<i>Paraphelenchus</i>	2	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0
<i>Aphelenchoides</i>	2	2.4	0.0	2.4	7.8	7.4	5.2	3.4	5.6
<i>Diphtherophora</i>	3	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0
<i>Tylencholaimus</i>	4	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Plant-parasites									
<i>Basiria</i>	2	2.2	3.5	13.9	10.2	9.6	15.7	6.4	5.4
<i>Tylenchus</i>	2	0.6	1.4	8.8	5.6	2.0	2.8	1.4	0.4
<i>Psilenchus</i>	2	1.2	0.6	3.4	2.6	1.8	3.6	17.7	14.4
<i>Paratylenchus</i>	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
<i>Merlinius</i>	3	0.4	0.4	1.4	0.4	0.0	0.0	0.0	0.2
<i>Helicotylenchus</i>	3	3.4	1.6	0.4	0.4	0.2	0.2	0.6	0.4
<i>Rotylenchus</i>	3	8.4	19.1	2.4	1.6	6.0	0.0	0.8	2.6
<i>Pratylenchus</i>	3	0.0	2.4	4.0	7.2	0.0	0.4	16.7	33.5
<i>Hirschmanniella</i>	3	0.0	0.0	0.2	0.0	1.0	2.6	0.6	0.6
Omnivore-predators									
<i>Monochromadora</i>	3	0.0	0.0	0.2	0.0	0.6	0.2	0.2	0.2
<i>Enchodelus</i>	4	3.4	8.7	4.0	2.8	4.6	7.6	7.0	4.6
<i>Dorydorella</i>	4	1.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0
<i>Microdorylaimus</i>	4	4.0	1.0	0.6	1.8	0.6	5.2	0.6	0.8
<i>Mesodorylaimus</i>	5	0.0	0.0	0.8	0.2	0.0	0.0	0.0	0.0
<i>Ecumenicus</i>	5	2.8	1.6	1.8	1.8	3.2	2.4	0.2	1.0
<i>Aporcelaimellus</i>	5	0.6	0.4	0.0	0.6	0.2	0.0	0.0	0.0
<i>Axonchium</i>	5	0.0	0.6	0.0	0.4	0.0	0.2	0.2	0.2
<i>Dorylaimellus</i>	5	0.2	0.4	0.6	0.2	0.0	0.2	0.2	0.0
<i>Discolaimium</i>	5	4.4	0.6	0.8	0.2	0.8	1.2	2.0	0.2
<i>Discolaimus</i>	5	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0

**Fig. 1.** The effect of cotton continuous cropping times and soil depth on the number of genera (a) and total nematodes (b). Error bars represent the standard error.

**Fig. 1.** Efecto de varios tiempos de cosechas continuas de algodón y profundidades del suelo en el número de géneros (a) y del total de nemátodos (b). Las barras de error representan el error estándar.



sities of BF and FF were 49 and 22 individuals/100 g dry soil, respectively, at that soil depth (Table 2). The highest and lowest value of PP were 196 (20-40 cm soil depth) and 39 individuals/100 g dry soil (0-20 cm soil depth), respectively (Table 2). The highest and lowest value of OP were 50 and 24 individuals/100 g dry soil, respectively, at 20-40 cm soil depth (Table 2).

Continuous cropping times and the interaction of continuous planting times and soil depths influenced the proportion of all trophic groups ( $P < 0.01$ ) (Table 2). Significant soil depth effects were observed on the proportion of FF and PP ( $P < 0.01$ ) (Table 2). The proportional contribution of BF individuals to total soil nematodes varied in the range of 14.4%-45.7%, with

the highest proportion in the 5-year continuous cropping at 20-40 cm soil depth, and the lowest proportion in the 20-year continuous cropping at 20-40 cm soil depth (Table 2). The proportional contribution of FF individuals to total soil nematodes varied in the range of 11.3%-36.7%, with the highest proportion in the 15-year continuous cropping at 0-20 cm soil depth, and the lowest proportion in the 5-year continuous cropping at 20-40 cm soil depth (Table 2). The proportion of PP individuals varied in the range of 16.2%-57.7%, with the highest proportion in the 20-year continuous cropping at 20-40 cm soil depth, and the lowest proportion in the 5-year continuous cropping at 0-20 cm soil depth (Table 2). Finally, the proportion of OP individuals varied in the range of 7.0%-17.0%, with the highest proportion in the 15-year continuous cropping at 20-40 cm soil depth, and the lowest proportion in the 20-year continuous cropping at 20-40 cm soil depth (Table 2).

**Nematode ecological indexes.** Continuous cropping times, soil depths and their interaction significantly influenced  $H'$ ,  $\lambda$ , WI, PPI, MI, PPI/MI, F/B and NCR ( $P < 0.0$  or  $P < 0.05$ ) (Table 3).

Diversity ( $H'$ ) was higher at the 10-year than 5-year continuous cropping at 0-20 cm soil depth, and at 15-year than the remaining continuous cropping times at 20-40 cm soil depth (Table 3). The lowest value appeared to be at the 5-year continuous cropping at 20-40 cm soil depth (Table 3). Simpson's Diversity index ( $\lambda$ ) had highest values at 5-year and 20-year continuous cropping at 20-40 cm soil depth (Table 3). Wasilewska index (WI) had lower values after 20-year continuous cropping than after the other continuous cropping times (Table 3). The highest and lowest values appeared in the 5-year continuous cropping at 0-20 cm soil depth, and the 20-year continuous cropping at 20-40 cm soil depth, respectively.

The Maturity index (MI) decreased ( $P < 0.01$ ) with increasing continuous cropping times (from 5- to 20-year) at both study depths (i.e., 0-20 cm and 20-40 cm soil depths). On the contrary, values of PPI and PPI/MI increased ( $P < 0.01$ ) as continuous cropping times also increased from 5-year to 20-year at both study soil depths (Table 3).

Values of NCR and F/B showed opposite trends when continuous cropping times increased from 5- to 20-year. When continuous cropping times decreased from 20- to 5-year at both soil depths of study, values increased ( $P < 0.01$ ) for NCR and decreased ( $P < 0.01$ ) for F/B (Table 3). Values of NCR at 5-year of continuous cropping at 0-20 cm (0.60) and 20-40 cm (0.80) soil depths indicated a faster bacterial-driven decomposition, while those at 20-year continuous cropping showed a slower fungal-driven decomposition. The ratio of the fungal to bacterial feeding nematode abundance indicated that the relative importance of the fungal and bacterial energy channels were responsive to years of continuous cropping (Table 3). The change trend of F/B values indicated the changes from a bacterial to a fungal decomposition pathway with increasing time of continuous cropping.

**Table 2.** Density (individuals per 100 g dry soil) and relative abundance (%) of nematode trophic groups in different continuous cropping times (years) of cotton and soil depths (mean  $\pm$  S.E.,  $n=5$ ).

**Tabla 2.** Densidad (individuos por 100 g de suelo seco) y abundancia relativa (%) de grupos tróficos de nematodos en diferentes tiempos de cosechas continuas (años) de algodón y profundidades de suelo (promedio  $\pm$  E.E.,  $n=5$ ).

	Soil depth (D)	Years (Y)	BF	FF	PP	OP
Density	0-20 cm	5	98.0 $\pm$ 5.3 b	65.0 $\pm$ 4.5 c	39.2 $\pm$ 3.0 e	39.6 $\pm$ 2.9 ab
		10	97.7 $\pm$ 3.6 b	86.1 $\pm$ 4.5 b	112.0 $\pm$ 5.3 c	28.5 $\pm$ 3.0 bc
		1	82.5 $\pm$ 1.8 c	93.2 $\pm$ 3.3 b	52.3 $\pm$ 3.4 e	26.0 $\pm$ 2.7 c
		20	76.8 $\pm$ 4.3 c	98.3 $\pm$ 3.4 ab	170.1 $\pm$ 6.1 b	40.0 $\pm$ 3.6 ab
	20-40 cm	5	87.1 $\pm$ 4.7 bc	21.8 $\pm$ 2.5 d	55.8 $\pm$ 5.9 de	26.8 $\pm$ 4.5 c
		10	150.3 $\pm$ 7.0 a	109.1 $\pm$ 8.3 a	114.8 $\pm$ 12.9 c	31.1 $\pm$ 7.7 bc
		15	79.6 $\pm$ 2.4 c	89.8 $\pm$ 4.3 b	75.1 $\pm$ 7.6 d	49.7 $\pm$ 3.8 a
		20	48.8 $\pm$ 2.5 d	71.2 $\pm$ 3.8 c	196.1 $\pm$ 8.6 a	23.7 $\pm$ 3.5 c
	ANOVA		P value			
	Y		<0.01	<0.01	<0.01	ns
	D		ns	<0.01	<0.01	ns
	Y $\times$ D		<0.01	<0.01	ns	<0.01
Relative density	0-20 cm	5	40.6 $\pm$ 2.2 b	26.8 $\pm$ 1.2 c	16.2 $\pm$ 1.2 f	16.4 $\pm$ 1.1 a
		10	30.1 $\pm$ 1.0 de	26.5 $\pm$ 1.4 c	34.5 $\pm$ 1.7 c	8.8 $\pm$ 0.9 c
		15	32.5 $\pm$ 0.7 cd	36.7 $\pm$ 1.0 a	20.6 $\pm$ 1.0 ef	10.2 $\pm$ 0.9 bc
		20	19.9 $\pm$ 1.0 f	25.5 $\pm$ 0.6 c	44.2 $\pm$ 1.9 b	10.3 $\pm$ 0.8 bc
	20-40 cm	5	45.7 $\pm$ 3.1 a	11.3 $\pm$ 1.1 e	29.0 $\pm$ 2.7 cd	13.9 $\pm$ 2.3 ab
		10	37.2 $\pm$ 1.7 bc	26.8 $\pm$ 1.1 c	28.0 $\pm$ 2.0 d	8.0 $\pm$ 2.3 c
		15	27.2 $\pm$ 1.0 e	30.5 $\pm$ 1.0 b	25.3 $\pm$ 1.8 de	17.0 $\pm$ 1.4 a
		20	14.4 $\pm$ 0.8 g	21.0 $\pm$ 1.1 d	57.7 $\pm$ 2.2 a	7.0 $\pm$ 1.0 c
	ANOVA		P value			
	Y		<0.01	<0.01	<0.01	<0.01
	D		ns	<0.01	<0.01	ns
	Y $\times$ D		<0.01	<0.01	<0.01	<0.01

Y: Continuous cropping times; D: Soil depth; BF: bacterivores; FF: fungivores; PP: plant-parasites; OP: omnivores-predators Density and proportions of each nematode trophic group in relation to continuous cropping times and soil depths.

Letters indicate significant differences among treatments.

## DISCUSSION

**Continuous cropping time and soil depth effects on nematode density and trophic groups.** The contribution of soil nematodes to ecosystem processes and functioning varies depending on the composition and diversity of the nematode community (Yeates & Bongers, 1999; Yeates et al., 2009; Costa et al., 2012; Porazinska et al., 2012). The trend of total nematode individuals in a continuous strawberry cropping soil increased firstly and then decreased, while the number of total individuals, bacterial-feeding and fungal-feeding nematodes, decreased with increasing time of continuous cropping; at the same time, the abundance of plant parasites increased with increasing continuous cropping times (Li et al., 2014). In our study, density

of total nematodes and trophic groups, except for densities of OP, clearly differed among different continuous cropping times ( $P<0.01$ ). However, the highest density of total nematodes was not found after 5 but after 10 years of planting. The number of fungivores showed an increasing trend with increasing time of continuous cropping while the number of bacterivores decreased at the same time. Soil depths did not show any effect on the density of nematodes. In agricultural systems, many management practices affect the soil environment which contributes to produce changes in the soil nematode community (Neher, 2001; Siddiqui & Akhtar, 2007). As time of continuous cropping increases, the physical and chemical characteristics of the soil (i.e., soil habitats) are changed, becoming more sensitive to soil disturbance. As a result, nematode taxonomic diversity

**Table 3.** Nematode ecological indexes of cotton continuous cropping years and soil depths.**Tabla 3.** Índices ecológicos de nematodos en varios años de cosechas continuas de algodón y profundidades de suelo.

Soil depth	Years	$H'$	$\lambda$	TD	PPI	MI	PPI/MI	WI	NCR	F/B
0-20 cm	5	2.32 bc	0.13 bc	3.42 b	0.45 f	1.82 a	0.25 d	4.29 a	0.60 b	0.67 d
	10	2.52 a	0.10 cd	3.44 b	0.76 cd	1.48 bc	0.53 c	1.62 cd	0.53 c	0.88 c
	15	2.36 ab	0.11 cd	3.40 b	0.48 f	1.80 a	0.27 d	3.42 b	0.47 d	1.13 b
	20	2.41 ab	0.12 cd	3.20 bc	1.07 b	1.36 c	0.80 b	1.04 de	0.44 de	1.29 b
20-40 cm	5	2.15 d	0.16 ab	3.01 c	0.82 c	1.46 bc	0.58 c	2.05 c	0.80 a	0.26 e
	10	2.34 b	0.13 bcd	3.34 b	0.66 de	1.61 b	0.42 cd	2.34 c	0.58 bc	0.73 cd
	15	2.52 a	0.10 d	3.81 a	0.54 ef	1.86 a	0.30 d	2.34 c	0.47 d	1.13 b
	20	2.17 cd	0.18 a	2.49 d	1.53 a	1.01 d	1.56 a	0.62 e	0.41 e	1.47 a
ANOVA		P value								
		Y	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
		D	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.05
		Y×D	<0.01	<0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

For each ecological index, letters indicate significant differences among treatments. Arrow directions indicate a trend for the values increasing from lowest to highest.

and the soil food web conditions will be influenced (Sánchez-Moreno, 2008). Therefore, it was not surprising that community composition and structure of soil nematodes showed fluctuations with increasing time from planting under the same planting pattern, irrigation and other management conditions. According to the number and proportion of total nematodes and trophic groups, our results indicated significant changes in the soil environment and nematode community structure after 20 years of continuous cotton cropping.

**Continuous cropping year and soil depth effects on nematode ecological indexes.** Nematodes are important components of soil ecosystems. Any changes in soil characteristics may result in changes in community dynamics of the nematodes, which can be assessed with use of ecological indexes (Tomar et al., 2009).

Shannon-Weiner diversity ( $H'$ ) and Simpson ( $\lambda$ ) indexes have been widely applied in nematodes (Yeates, 1984; Wasilewska, 1979; Boucher, 1990; Lambshead et al., 1983; Tietjen, 1989). While the Shannon diversity index ( $H'$ ) gives more weight to rare species, the Simpson index ( $\lambda$ ) gives more weight to common species (Liang et al., 2002; Pen-Mouratov et al., 2003). Some ecological indexes showed fluctuation trends following the increase of continuous cropping times in our study. The Shannon index was higher in the 10- than in the other-year cotton fields, whereas the Simpson index was lowest in the 10-year cotton field. The combination of these two indexes indicated that nematode diversity was higher in the 10-year situation.

The Maturity (MI) and Plant Parasite (PPI) indexes were useful to reflect the degree of disturbance in the soil ecosystem (Bongers, 1990). The higher MI values indicated a higher presence of persisting free-living nematodes, and lower val-

ues indicated the presence of colonizer free-living nematodes. Higher PPI values indicated a higher presence of persisting plant-feeding nematodes (Thakur et al., 2014). The MI decreased after disturbance (Ettema & Bongers, 1993). Five- and 15-year soils at 0-20 cm, and the 15-year soil at 20-40 cm had a higher MI value, and a lower PPI value, than the other soils in our research ( $P < 0.01$ ). This suggests that the soil was comparatively less disturbed than the other soils. Zheng et al. (2011) reported higher MI and lower PPI on 0-year than continuous tomato cropping soils, and an increase of plant species-specific feeding nematode abundance. For example, more abundant *Meloidogyne* spp. populations led to higher PPI in continuous cropping soil where the long-term presence of the same host plant provided a food source to the nematodes. In our study, PPI and MI stabilized at the different soil layers after 10 and 15 years of continuous cropping. The PPI was lower at 0-20 cm than at 20-40 cm soil depth after 5 and 20 years of continuous cropping. The MI was higher at 0-20 cm than at 20-40 cm soil depth in the 5-year and 20-year continuous cropping. Bongers et al. (1997) suggested that the ratio PPI/MI would indicate changes in soil fertility and environmental quality, and that farming and other agricultural disturbances could improve the PPI/MI value of soil nematodes. We found a higher PPI/MI value after 20-year than the other continuous cropping times. This indicates that disturbance was strong after 20 years of continuous planting.

The WI, F/B and NCR were calculated by the abundance of three trophic groups (excluding omnivores-predators). The WI was the ratio between the abundance of microbial-feeding (FF + BF) and plant-feeding (PP) nematodes [(FF + BF)/PP]. A ratio higher than 1 indicates positive effects of soil nematodes on plant productivity (i.e., a relative decline in



plant-feeding nematodes), whereas values  $<1$  stand for negative effects on plant biomass production (Wasilewska, 1997). The highest and lowest values of WI appeared in the 5-year continuous cropping at 0–20 cm (4.29), and the 20-year continuous cropping at 20–40 cm (0.62) soil depth, respectively. The number and proportion of plant parasites were higher after 20-year than other continuous times of cropping. This shows that plant parasites effects on plant biomass production were greater after 20 years of continuous cropping.

Decomposition processes in the soil, although ultimately dependent on the plant resource base, are often allocated to either the bacterial-based energy channel (or pathway) or the slower fungal-based channel (Moore & Hunt, 1988). The relative importance of energy and nutrient flow through the bacterial and fungal channel in soil could be assessed by the fungal to the bacterial feeder ratio (F/B) (Twinn, 1974; Wasilewska, 1979; Freckman & Ettema, 1993). We found that F/B ranged from 0.26 to 1.47, and that F/B values increased with increasing continuous cropping times. This showed that the decomposition pathway changed from bacterial to fungal. The nematode channel ratio (NCR) is an important indicator of the decomposition pathway in the detritus food webs, and it can achieve values between 1 (totally bacterial-mediated) and 0 (totally fungal-mediated) (Yeates et al., 2003; Jiang et al., 2007). The values of NCR were sensitive to the effects of cropping times and soil depths, ranging from 0.41 to 0.80, with higher values after 5- than the other-year situations. This indicated that the bacterial decomposition pathway was more important in the 5-year situation, and played a more important role in nutrient cycling. A decreasing trend of NCR was found with increasing cropping times. In summary, the F/B and NCR showed that bacterial decomposition was the dominant pathway in the 5-year continuous cropping, while fungal decomposition was the dominant pathway after 20-year. The relative importance of the fungal pathway often reflected lower rates of decomposition (Yeates & Boag, 2004).

## CONCLUSIONS

Continuous cotton cropping played an important role in determining the composition and diversity of the soil nematode community. The responses of soil nematodes were more sensitive to continuous cropping than soil depth, which may influence the decomposition processes of organic matter in agro-ecosystems. The numbers of total nematodes and trophic groups,  $H'$ ,  $\lambda$ , WI, PPI, MI, PPI/MI, F/B and NCR had significant differences among the study treatments. Some ecological indexes and the number of trophic groups appeared to fluctuate with the increase of continuous cropping years. This showed that continuous cropping of cotton had unstable effects on the soil environment. The present study showed that changes of soil nematode communities and trophic groups could indicate changes in the soil environment and the nematode community structure with changes of continuous cropping times.

## ACKNOWLEDGEMENTS

This research was supported by the National Natural Science Foundation of China (41161044 and 31400459).

## REFERENCES

- Ahmad, W. & M.S. Jairjuri (2010). Mononchida: the Predaceous Nematodes. In: E.J. Brill (ed.). pp.1–299. Nematology Monographs and Perspectives, vol. 7, Leiden, The Netherlands.
- Bongers, T., (1990). The maturity index: an ecological measure of environmental disturbance based on nematode species composition. *Oecologia* 83: 14–19.
- Bongers, T., R.G.N. de Goede, G.W. Korthals, G.W. Yeates (1995). Proposed changes of c-p classification for nematodes. *Russian Journal of Nematology* 3: 61–62.
- Bongers, T., H. van der Meulen & G. Korthals (1997). Inverse relationship between the nematode maturity index and plant parasite index under enriched nutrient conditions. *Applied Soil Ecology* 6: 195–199.
- Bongers, T. & M. Bongers (1998). Functional diversity of nematodes. *Applied Soil Ecology* 10: 239–251.
- Bongers, T. & H. Ferris (1999). Nematode community structure as a bioindicator in environmental monitoring. *Trends in Ecology & Evolution* 14: 224–228.
- Boucher, G. (1990). Patterns of nematode species diversity in temperate and tropical subtidal sediments. *Marine Ecology* 11:133–146.
- Costa, S.R., B.R. Kerry, R.D. Bardgett & K.G. Davies (2012). Interactions between nematodes and their microbial enemies in coastal sand dunes. *Oecologia* 170: 1053–1066.
- Ettema, C.H. & T. Bongers (1993). Characterization of nematode colonization and succession in disturbed soil using the maturity index. *Biology & Fertility of Soils* 16: 79–85.
- Ferris, H., T. Bongers & R.G.M. de Goede (2001). A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. *Applied Soil Ecology* 18: 13–29.
- Ferris, H. & M.M. Matute (2003). Structural and functional succession in the nematode fauna of a soil food web. *Applied Soil Ecology* 23: 93–110.
- Freckman, D.W. & C.H. Ettema (1993). Assessing nematode communities in agroecosystems of varying human intervention. *Agriculture Ecosystems & Environment* 45: 239–261.
- Fu, S.L., D.C. Coleman, P.F. Hendrix & D.A. Crossley (2000). Responses of trophic groups of soil nematodes to residue application under conventional tillage and no-till regimes. *Soil Biology & Biochemistry* 32: 1731–1741.
- Jairajpuri, M.S. & W. Ahmad (1992). Dorylaimida: Free Living, Predaceous and Plant Parasitic Nematodes. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi.
- Jiang, D.M., Q. Li, F.M. Liu, Y. Jiang & W.J. Liang (2007). Vertical distribution of soil nematodes in an age sequence of *Caragana microphylla* plantations in the Horqin Sandy Land, Northeast China. *Ecological Research* 22: 49–56.
- Jin, J.X. (2008). Xinjiang Statistical Yearbook. Beijing: China Statistics Press, pp 280–308.
- Lambshead, P.J.D., H.M. Platt & K.M. Shaw (1983). The detection of differences among assemblages of marine benthic species based on an assessment of dominance and diversity. *Journal of Natural History* 17: 859–874.



- Li H.Q., Q.Z. Liu, L.L. Zhang, Y.L. Wang, H. Zhang, P.H. Bai & X.B. Luan (2014). Accumulation of phenolic acids in the mono-cultured strawberry soils and their effect on soil nematodes. *Chinese Journal of Ecology* 33: 169-175.
- Liang, W.J., S. Mouratov, Y. Pinhasi-Adiv & Y. Steinberger (2002). Seasonal variation in the nematode communities associated with two halophytes in a desert ecosystem. *Pedobiologia* 46: 63-74.
- Liang, W.J., Q. Li, Y. Jiang & D.A. Neher (2005). Nematode faunal analysis in an aquatic brown soil fertilized with slow-release urea, Northeast China. *Applied Soil Ecology* 29: 185-192.
- Liang, W.J., S. Zhong, J.F. Hua, C.Y. Cao & Y. Jiang (2007). Nematode faunal response to grassland degradation in Horqin Sandy Land. *Pedosphere* 17: 611-618.
- Liang, W.J., Y.L. Lou, Q. Li, S. Zhong, X.K. Zhang & J.K. Wang (2009). Nematode faunal response to long-term application of nitrogen fertilizer and organic manure in Northeast China. *Soil Biology and Biochemistry* 41: 883-890.
- Moore, J.C. & H.W. Hunt (1988). Resource compartmentation and the stability of real ecosystems. *Nature* 333: 261-263.
- Neher, D.A. (2001). Role of nematodes in soil health and their use as indicators. *Journal of Nematology* 33: 161-168.
- Ni, T.Q., C.Y. Tian & W.K. Hu (2002). Some vital problems in cotton production in Xinjiang and the counter-measures for achieving a sustainable development. *Arid Zone Research* 19: 57-61.
- Okada, H. & H. Harada (2007). Effects of tillage and fertilizer on nematode communities in a Japanese soybean field. *Applied Soil Ecology* 35: 528-598.
- Pen-Mouratov, S., M. Rakhimbaev & Y. Steinberger (2003). Seasonal and spatial variation in nematode communities in a Negev desert ecosystem. *Journal of Nematology* 35: 157-166.
- Pen-Mouratov, S., N. Shukurov & Y. Steinberger (2010). Soil free-living nematodes as indicators of both industrial pollution and livestock activity in Central Asia. *Ecological Indicators* 10: 955-967.
- Porazinska, D.L., R.M. Giblin-Davis, T.O. Powers & W.K. Thoma (2012). Nematode spatial and ecological patterns from tropical and temperate rainforests. *Plos One* 7: e44641. doi:10.1371/journal.pone.0044641.
- Powell, J.R. (2007). Linking soil organisms within food webs to ecosystem functioning and environmental change. *Advances in Agronomy* 96: 307-350.
- Ruess, L., A. Michelsen & S. Jonasson (1999). Simulated climate change in subarctic soils: responses in nematode species composition and dominance structure. *Nematology* 1: 513-526.
- Sánchez-Moreno, S., S. Smukler, H. Ferris, A.T. O'Geen & L.E. Jackson (2008). Nematode diversity, food web condition, and chemical and physical properties in different soil habitats of an organic farm. *Biology and Fertility of Soils* 44: 727-744.
- Shannon, C.E. (1948). A mathematical theory of communication. *Bell System Technical Journal* 27: 379-423.
- Siddiqui, Z.A. & M.S. Akhtar (2007). Effects of AM fungi and organic fertilizers on the reproduction of the nematode *Meloidogyne incognita* and on the growth and water loss of tomato. *Biology and Fertility of Soils* 43: 603-609.
- Simpson, E.H. (1949). Measurement of diversity. *Nature* 163: 668.
- Statistical bureau of Xinjiang (2010). Xinjiang Statistical Yearbook. Beijing: China Statistics Press, pp 322-333.
- Thakur, M.P., P.B. Reich, N.A. Fisichelli, A. Stefanski, S. Cesarz, T. Dobies, R.L. Rich, S.E. Hobbie & N. Eisenhauer (2014). Nematode community shifts in response to experimental warming and canopy conditions are associated with plant community changes in the temperate-boreal forest ecotone. *Oecologia* 175: 713-723.
- Tietjen, J.H. (1989). Ecology of deep-sea nematodes from the Puerto Rico Trench area and Hatteras Abyssal Plain. *Deep Sea Research* 36: 1579-1594.
- Tomar, V.V.S. & W. Ahmad (2009). Food web diagnostics and functional diversity of soil inhabiting nematodes in a natural woodland. *Helminthologia* 46: 183-189.
- Twinn, D.C. (1974). Nematodes. In: C.H. Dickinson and G.J.F. Pugh (eds), pp. 421-465. *Biology of Plant Litter Decomposition*. Academic Press, London.
- Walag, A.M.P. & M.O.P. Canencia, (2016). Physico-chemical parameters and macrobenthic invertebrates of the intertidal zone of Gusa, Cagayan de Oro City, Philippines. *AES Bioflux* 8: 71-82.
- Wang, K.H., R. McSorley & R.N. Gallaher (2009). Can nematode community indices elucidate plant health conditions? *Journal of Nematology* 41: 392.
- Wasilewska, L. (1979). The structure and function of soil nematode communities in natural ecosystems and agroecosystems. *Polish Ecological Studies* 5: 97-145.
- Wasilewska, L. (1997). Soil invertebrates as bioindicators, with special reference to soil inhabiting nematodes. *Russian Journal of Nematology* 5: 113-126.
- Wu, H.Y. & L.B. Shi (2011). Effects of continuous cropping duration on population dynamics of second-stage juvenile *Meloidogyne* spp. and free-living soil nematodes. *African Journal of Agricultural Research* 6: 307-312.
- Wu, J.H., C.Z. Fu, S.S. Chen & J.K. Chen (2002). Soil faunal responses to land use: effect of estuarine tideland reclamation on nematode communities. *Applied Soil Ecology* 21: 131-147.
- Yeates, G.W. (1984). Variation in soil nematode diversity under pasture with soil and year. *Soil Biology & Biochemistry* 16: 95-102.
- Yeates, G.W., T. Bongers, R.G.M. de Goede, D.W. Freckman & S.S. Georgieva (1993). Feeding habits in soil nematode families and genera—an outline for soil ecologists. *Journal of Nematology* 25: 315-331.
- Yeates, G.W., K.R. Tate & P.C.D. Newton (1997). Response of the fauna of a grassland soil to doubling of atmospheric carbon dioxide level. *Biology and Fertility of Soils* 25: 305-317.
- Yeates, G.W. & T. Bongers (1999). Nematode diversity in agroecosystems. *Agriculture, Ecosystems & Environment* 74: 113-135.
- Yeates, G.W. (2003). Nematodes as soil indicators: functional and biodiversity aspects. *Biology and Fertility of Soils* 37: 199-210.
- Yeates, G.W., P.C.D. Newton & D.J. Ross (2003). Significant changes in soil microfauna in grazed pasture under elevated carbon dioxide. *Biology and Fertility of Soils* 37: 199-210.
- Yeates, G.W. & B. Boag (2004). Background for nematode ecology in the 21st century. In: Chen, Z.X., Chen, S.Y., Dickson, D.W., (eds), pp 424-425. *Nematology advances and perspectives*. Vol. I: Nematode morphology, physiology and ecology. CABI, Wallingford, UK.
- Yeates, G.W., H. Ferris, T. Moens & W.H. van der Putten (2009). The role of nematodes in ecosystems. In: Wilson, M.J., Kakouli-duarte, T. (eds), pp. 1-44. *Nematodes as Environmental Bioindicators*. CABI, Wallingford, UK.
- Zheng, G.D., L.B. Shi, H.Y. Wu & D.L. Peng (2011). Nematode communities in continuous tomato cropping field soil infested by root-knot nematodes. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science*, pp. 1-8.